Amendment To The Claims

1. (Currently Amended) A method for screening for agents that affect protein degradation rates, the method comprising:

~~taking~~ i) expressing a fusion protein in each cell within a library of cells, the ~~cells expressing a~~ fusion protein comprising a reporter protein and a protein encoded by a sequence from a cDNA library derived from a sample of cells, the sequence from the cDNA library varying within the cell library;

ii) inhibiting further expression of the fusion protein to allow the expressed fusion protein to degrade in the cell;

iii) selecting a population of cells from the library of cells based on the population of cells having different reporter signal intensities than other cells in the library, the difference being indicative of the population of cells expressing shorter lived fusion proteins than the fusion proteins expressed by the other cells in the library;

iv) contacting the ~~library~~ selected population of cells from step iii) with a plurality of agents which may affect protein degradation rates;

v) for each agent, selecting cells in the ~~library~~ selected population from step iv) ~~which express short-lived proteins~~ based on whether the cells have different reporter signal intensities than ~~other~~ the cells in the ~~library~~ selected population of cells from step iii) without being contacted with the agent, the difference being indicative of the selected cells expressing ~~shorter~~ short lived fusion proteins whose degradation is affected by the agent ~~than the fusion proteins expressed by the other cells in the library~~; and

characterizing the fusion proteins expressed by the selected cells for each agent.

2. (Original) A method according to claim 1, wherein the method further comprises comparing which fusion proteins are expressed by the selected cells for each agent.

3. (Currently Amended) A method for monitoring effects different growth conditions have on expression of short-lived proteins, the method comprising:

exposing samples of cells to different growth conditions;

forming cDNA libraries from the sample of cells after exposure to the different growth conditions;

forming a library of cells for each cDNA library, the cells in the library expressing a fusion protein comprising a reporter protein and a protein encoded by a sequence from the cDNA library derived from a sample of cells, the sequence from the cDNA library varying within the cell library;

for each library of cells,

inhibiting further expression of the fusion protein to allow the expressed fusion protein to degrade in the cell,

identifying cells within the library that express fusion proteins that are degraded *in vivo* more rapidly than other fusion proteins, and

characterizing fusion proteins expressed by the identified cells; and

comparing which fusion proteins are characterized for each library of cells, differences in the characterized fusion proteins indicating differences in the short-lived proteins expressed by when the cells are exposed to the different ~~agents~~ growth conditions.

4. (Original) A method according to claim 3, wherein exposing the samples of cells to different conditions comprises exposing the cells to different agents.

5. (Currently Amended) A method according to claim 3, wherein identifying cells within the library that express fusion proteins that are degraded *in vivo* more rapidly than other fusion proteins comprises

~~modifying a rate of protein expression or degradation by the cells, and~~

selecting a population of the cells based on whether the cells have different reporter signal intensities than other cells after the rate of protein expression or degradation has been modified, the difference being indicative of the selected population of cells expressing shorter lived fusion proteins than the fusion proteins expressed by the other cells in the library.

6. (Currently Amended) A method for monitoring effects different growth conditions have on expression of short-lived proteins, the method comprising:

exposing samples of cells to different conditions;

forming cDNA libraries from the sample of cells after exposure to the different growth conditions;

forming a library of cells for each cDNA library, each cell in the library expressing a fusion protein comprising a reporter protein and a protein encoded by a sequence from the cDNA library derived from a sample of cells, the sequence from the cDNA library varying within the cell library;

for each library of cells,

partitioning the library of cells into populations of cells based on an intensity of a reporter signal from the fusion protein such that cells partitioned into a given population have a reporter signal within a desired range of reporter signal intensity,

expressing the fusion proteins in the given population of cells,

inhibiting further expression of the fusion protein in the given population of cells to allow the expressed fusion protein to degrade in the cell;

~~modifying a rate of protein expression or degradation by the cells for a given population of cells,~~

selecting a subpopulation of the cells from the given population of cells based on whether the cells have a different reporter signal intensity than the other cells in the given population, the difference being indicative of the selected subpopulation of cells expressing shorter lived fusion proteins than the fusion proteins expressed by the other cells in the given population,

characterizing fusion proteins expressed by at least a portion of the selected cells; and

comparing which fusion proteins are characterized for each library of cells, differences in the characterized fusion proteins indicating differences in the short-lived proteins expressed by when the cells are exposed to the different ~~agents~~ different growth conditions.

7. (Original) A method according to claim 6 wherein exposing the samples of cells to different conditions comprises exposing the cells to different agents.

8. (Currently Amended) A method for screening for differences in short-lived proteins expressed by first and second cell samples, the method comprising:

forming cDNA libraries for first and second samples of cells;

forming a library of cells for each cDNA library, the cells in the library expressing a fusion protein comprising a reporter protein and a protein encoded by a sequence from the cDNA

library derived from a sample of cells, the sequence from the cDNA library varying within the cell library;

for each library of cells,

inhibiting further expression of the fusion protein to allow the expressed fusion protein to degrade in the cell,

identifying cells within the library that express fusion proteins that are degraded *in vivo* more rapidly than other fusion proteins, and

characterizing fusion proteins expressed by the identified cells; and

comparing which fusion proteins are characterized for each library of cells, differences in the characterized fusion proteins indicating differences in the short-lived proteins expressed by the first and second samples cells.

9. (Currently Amended) A method for screening for differences in short-lived proteins expressed by first and second cell samples, the method comprising:

forming cDNA libraries for first and second samples of cells;

forming a library of cells for each cDNA library, the cells in the library expressing a fusion protein comprising a reporter protein and a protein encoded by a sequence from the cDNA library derived from a sample of cells, the sequence from the cDNA library varying within the cell library;

for each library of cells,

[[`]] partitioning the library of cells into populations of cells based on an intensity of a reporter signal from the fusion protein such that cells partitioned into a given population have a reporter signal within a desired range of reporter signal intensity,

expressing the fusion proteins in the given population of cells,

inhibiting further expression of the fusion protein in the given population of cells to allow the expressed fusion protein to degrade in the cell;

~~modifying a rate of protein expression or degradation by the cells for a given population of cells,~~

selecting a subpopulation of the cells based on whether the cells have different reporter signal intensities than the other cells after the rate of protein expression or degradation has been modified, the difference being indicative of the selected

subpopulation of cells expressing shorter lived fusion proteins than the fusion proteins expressed by the other cells in the given population, and

characterizing fusion proteins expressed by at least a portion of the selected cells; and

comparing which fusion proteins are characterized for each library of cells, differences in the characterized fusion proteins indicating differences in the short-lived proteins expressed by the first and second samples cells.

10. (New) A method according to claim 1, wherein inhibiting further expression of the fusion protein includes inhibiting further synthesis of the fusion protein.

11. (New) A method according to claim 10, wherein the further synthesis of the fusion protein is inhibited by adding cycloheximide to the cell.

12. (New) A method according to claim 1, wherein the reporter protein is a fluorescent protein.

13. (New) A method according to claim 1, wherein the reporter protein is a green fluorescence protein (GFP) or enhanced green fluorescence protein (EGFP).

14. (New) A method according to claim 8, wherein inhibiting further expression of the fusion protein includes inhibiting further synthesis of the fusion protein.

15. (New) A method according to claim 14, wherein the further synthesis of the fusion protein is inhibited by adding cycloheximide to the cell.

16. (New) A method according to claim 8, wherein the reporter protein is a fluorescent protein.

17. (New) A method according to claim 8, wherein the reporter protein is a green fluorescence protein (GFP) or enhanced green fluorescence protein (EGFP).

REMARKS

The present Amendment is in response to the Examiner's Non-Final Office Action mailed on March 20, 2003. Claims 1, 3, 5, 6, 8, and 9 have been amended. New claims 10-17 are added. Claims 1-17 are now pending.

Reconsideration of the application is respectfully requested in view of the above amendments to the claims and the following remarks. For the Examiner's convenience and reference, Applicants' remakes are presented in the order in which the corresponding issued were raised in the Office Action.

I. Rejections Under 35 U.S.C. §102(b)

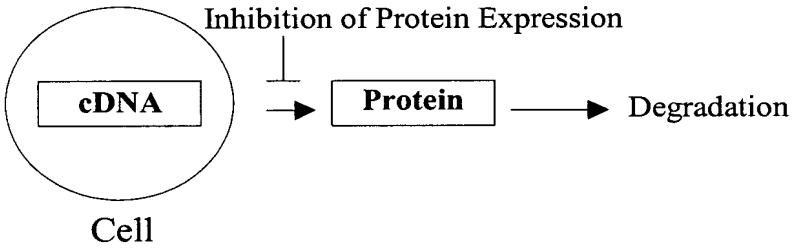
The Examiner rejected claims 1-9 under 35 U.S.C. §102(b), as being anticipated by O'Hare et al. (PCT International Publication No. WO 00/08182, February 17, 2000). Specifically, the Examiner asserts that O'Hare et al. teaches the methods for 1) screening agents that affects protein degradation rates (Abstract), 2) wherein exposing the samples of cells to different conditions comprises exposing the cells to different agents (Page 2, line 4 to page 3, line 24 and claim 7), 3) partitioning the library of cells into populations of cells based an intensity of a reporter signal from the fusion protein such that the cells partitioned into a given population have a reporter signal within a desired range of reporter signal intensity (Page 5, line 26 to page 12, line 23), and 4) screening for differences in short-lived proteins expressed by first and second cell samples (Page 7, line 15 to page 8, line 7). Applicants respectfully traverse the Examiner's rejection based on the following reasons.

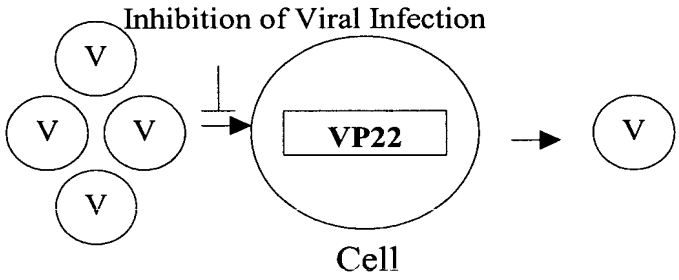
Independent claims 1, 3, 6, 8, and 9 as amended each specify a method for selecting **cells** based on whether the cells express a short-lived protein. Each of the claimed methods is a high throughput assay for screening a library of cells within which **a library of fusion proteins** (e.g., GFP fusion proteins) are expressed. According to the method, the library of fusion proteins are **expressed first and then allowed to degrade** in the cells. To monitor the degradation of the expressed fusion proteins, **further expression** of the fusion protein is inhibited, for example, by addition of a protein translation inhibitor, cycloheximide. Page 20, lines 1-3. The claimed methods distinctly differ from O'Hare et al. in numerous aspects.

First, the claimed methods utilize a **library of cells**, each of which expresses a fusion protein encoded by a **cDNA library**. The sequence encoding the fusion protein varies within the

cDNA library. In contrast, O'Hare et al. teaches construction of a recombinant virus encoding a **single** herpesviral structural protein, VP22, fused with GFP. *See* "Abstract" and page 1, lines 16-24. This recombinant virus was used to inoculate cells. Nowhere does O'Hare et al. teach or suggest constructing **a library of cells expressing a library of different fusion proteins**.

Second, the claimed method specifies the steps of expressing the fusion protein and inhibiting **further expression** of the fusion protein so as to monitoring **the degradation of the expressed protein**. In contrast, O'Hare et al. teaches **inhibition of viral infection** by using neutralizing antibody or inhibitors of **infection** of cells by virus. By its parasitic nature, without infection of the cells the virion (V) would not be able to express the viral proteins encoded thereby. A figure shown below exemplarily and schematically illustrates the differences between these two modes of expression.





As illustrated by this figure, the claimed methods monitor the degradation of the fusion proteins after they are first expressed whereas O'Hare et al teaches inhibition of viral infection by blocking infusion of virion into the cell membrane. Thus, O'Hare et al. fails to teach the steps of allowing the fusion protein to be synthesized first and then inhibiting further synthesis in order to monitoring degradation of the synthesized protein.

Third, the claimed methods specify a step of **selecting a population of cells** which express short-lived proteins from the library of cells. This selection is based on the difference between the reporter signal intensities of the selected population and those of other cells in the library. For example, if GFP is used as the reporter protein, the population of cells is selected

based on the lowered fluorescence intensities of GFP than those of other cells in the library. In contrast, O'Hare et al. teaches **selecting viral plaques** that exhibited green fluorescence in an agarose plate and then used the selected plaques to inoculate cells. Page 7, lines 29-32. Thus, O'Hare et al. does not even teach the claimed step of selecting a population of cells, let alone teaching the selection of cells expressing short-lived proteins.

In view of the numerous distinct differences between the claimed methods and the method disclosed in O'Hare et al., the cited reference fails to teach every element of the claims and thus fails to anticipate the claimed invention under 35 U.S.C. § 102(b). Withdrawal of the rejection is therefore respectfully requested.

CONCLUSION

Applicants believe that they are entitled to a letters patent, and respectfully solicit the Examiner to expedite prosecution of this patent to issuance. Should the Examiner have any questions, Examiner is encouraged to telephone the undersigned.

Respectfully,
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